Senna alata: Phytochemistry, Antioxidant, Thrombolytic, Anti-inflammatory, Cytotoxicity, Antibacterial activity, and GC-MS analysis

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ABSTRACT

Objective: Nepal's medicinal herbs are rich in cultural importance and have several uses. Senna alata, a plant belonging to the Leguminosae family, is prized for its aesthetic and therapeutic qualities. The goal of the study was to extract Senna alata leaves using several solvent macerations.

Methods: The study aims to evaluate the phytochemistry, total phenolic and flavonoid levels, antioxidant qualities in vitro, anti-inflammatory effects, cytotoxicity, anti-thrombolytic potential, and antibacterial activity, a variety of methodologies were employed.

Results: The extractive values of Senna alata were determined as 1.58%, 0.78%, and 5.92% in hexane, ethyl acetate, and methanol, respectively. GC-MS analysis revealed major compounds such as 3-Methylmannoside, Neophytadiene, Campesterol, and Vitamin E in the leaf extract. Qualitative phytochemical screening confirmed the presence of tannins, carbohydrates, flavonoids, cardiac glycosides, glycosides, and saponins in the methanol extract. The total phenolic and flavonoid contents were 46.36±4.5 mg GAE/g and 480.4±3.055 QE/g of dried extract, respectively. The extract exhibited significant antioxidant and anti-inflammatory activities, with IC50 values of 29.81 and 9.93, respectively. Additionally, it demonstrated cytotoxic activity with an LC50 value of 767.85 in the brine shrimp bioassay. In terms of thrombolytic activity, the extract showed clot lysis percentages of 7.89% and 10.13% at concentrations of 10 mg/ml and 25 mg/ml, respectively.

Conclusion: The methanolic extract of Senna alata leaves displayed therapeutic potential, including antioxidant, anti-inflammatory, cytotoxic, thrombolytic, and antibacterial effects. The presence of several bioactive compounds, as confirmed by GC-MS analysis, further supports the plant's potential for therapeutic use.

Keywords: *Senna alata*, phytochemical screening, antioxidant activity, anti-inflammatory activity, thrombolytic activity, cytotoxic activity

INTRODUCTION

Due to a lack of research and scientific validation, herbal medicines are still not widely accepted by the medical community [1]. Antibiotic resistance is an issue in today's world because of the widespread use of commercial antibiotics to treat infectious diseases [2].

The immune system's natural reaction to harm caused by microbial, chemical, and physical agents is inflammation. Excessive swelling leads too many acute and long-term illnesses such as autoimmune diseases,

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illnesses of the circulatory system, malignancies, and metabolic and neurological disorders [3].

An entire class of extremely reactive molecules produced by the metabolism of oxygen is known as reactive oxygen species (ROS). Normally occurring physiological quantities of ROS are necessary for cellular functions. Antioxidants, both natural and synthetic, can help the body reduce oxidative damage brought on by reactive oxygen species [3].*Senna alata* contains chemical components that have been shown to have a variety of pharmacological activities. However, the real effects are yet unknown, thus more research is needed to investigate its medical benefits [4].

Meantime, some studies reported that the generation of reactive oxygen species (ROS) caused inflammation which has been related to the pathogenesis factors for various diseases in patients [5]. Hence, this complication may be prevented through the scavenge ROS by the free radical scavenging mechanism. Most bioactive phytoconstituents like; phenolic, alkaloid, and flavonoid compounds curing the endogenous cells and cellular proteins through the free radical scavenging activity [6-8]. Such preventive effects are important for inflammation, cytotoxicity, and microbial disease which is also due to oxidative processes.

Senna alata is commonly known as a candle tree or ringworm brush [1]. It is locally known as the Agasti plant and is used in various religious rituals. *Senna alata* is a significant flowering plant that is both decorative and medicinal [9]. Foliage of plants is utilized as a pungent, expectorant, vermicide, purgative, and as well as in the handling of fungal illnesses. *Cassia alata* leaf extract may have cytotoxic, analgesic, antibacterial, anti-inflammatory, and fungicidal properties [1, 9].

The search for new antimicrobial active agents obtained by using plant extracts of *Senna alata* has led to the discovery of many clinically useful drugs, which help to solve the problems of antibiotic resistance exhibited by pathogenic microorganisms [2]. *Senna alata* contains anti-inflammatory agents; hence can be a sensible and successful research approach in the hunt for novel anti-inflammatory medications. These plant extracts may led to the concentration of scientific effort on finding reliable and efficient sources of antioxidants that prevent the cellular and tissue damage caused by oxidative stress [3].

In the search for new drugs, the search for bioactive plant components from natural sources is always a deciding factor. Research on *Senn aalata* leaves will improve the proper use of this plant in various medical conditions as an alternative treatment plan and will help to find possible therapeutic agents for specific diseases. The purpose of this study is to look into the in vitro antioxidant, antibacterial, anti-inflammatory, thrombolytic, cytotoxic effects, phytochemical profiling, and GC-MS analysis of *Senna alata* leaf extract.

MATERIAL AND METHODS

Drugs and chemicals

Methanol, Ethyl acetate, and Hexane were purchased from Merck Life Science Pvt. Ltd., while the Vincristine Sulphate was obtained from the Neon Laboratories, Limited, India. Standard reference drugs Diclofenac and Ciprofloxacin were obtained from Saheka India and Arati Drugs India respectively. Ascorbic acid, Gallic acid, Quercetin, and DPPH was purchased from Hi-Media, India. Every reagent and chemical of analytical are used for this research.

Study plant material

Senna alata leaves were gathered from Chandragiri-06, Kathmandu, Nepal. Plant herbarium was authenticated by a taxonomist from National Herbarium and Plant Laboratories, Kathmandu, Nepal. A voucher specimen (Acc. No. 02-1221- 2020) was deposited in the herbarium of Manmohan Memorial Institute of Health Science for future reference.

Plant extract preparation and extract percentage yield

Fresh water was used to clean the plant leaves completely. After that, the leaves were dried and

sliced into little bits and introduced to successive maceration techniques in which 3 different solvents were used according to the polarity of the solvents. The method involved the use of non-polar solvent ethyl acetate and n-hexane and polar solvent methanol for extraction of active constituents from the powdered leaves. For this, a known amount of powdered sample was taken in a beaker. First, hexane, then ethyl acetate, and finally methanol was used for extraction in the polarity order using the maceration process. Ultimately, a table fan was used to concentrate the extracted material at room temperature after it had been moved to a stainless-steel plate. Dried extracts were stored in borosilicate glass vials and subjected to different investigations. Then obtained extract was filtered with Whatman No. 1 filter paper. The filtrate was evaporated by a rota evaporator under reduced pressure (60 mmHg) at 40ºC and stored at 4ºC. The extract yield percentage can be determined using the formula below:

Extract % yield = (Weight of dried extracts / Weight of plants sample) ^{x}100

GC-MS analysis

The chemical components present in the methanolic extract that demonstrated the different biological activities were identified by performing a GC-MS analysis on the extract. The Nepal Academy of Science and Technology in Khumaltar, Lalitpur, conducted the GC-MS. For the GC-MS examination of plant material, GCMS QP2010 (Shimadzu, Kyoto, Japan) equipped with RTx-5MS fused silica capillary column of 30m length X 0.25 mm diameter X 0.25µm film thickness. Helium (>99.99% purity) with 36.2 cm/sec linear velocity was employed as carrier gas. The system was configured using 3.9 ml/min of total flow rate, 0.95 ml/min of column flow, and 3.0 ml/min purge flow. The volume of the injected sample was 1µl. The injector was set in splitless mode having 280ºC of temperature. Starting at 100ºC, the oven temperature increased to 250ºC at 15ºC/min with a 1-minute pause. It then increased to 280ºC at 30ºC/min with a 1-min hold, then it increased once more from 280ºC to 300ºC at 15ºC/min with an 11-minute hold. With solvent cut-off duration of 3.5 minutes, the ion source and interface temperatures were set at 200ºC and 280ºC, respectively. 20 minutes were spent on the mass range scan, which covered 40 to 500 m/z. By comparing the mass spectra of the compounds with information from the NIST 08 mass spectral collection, the compounds were identified.

Qualitative phytochemical analysis

The *Senna alata* leaves extracts undergo phytochemical analysis for the detection of plant secondary metabolites like alkaloid, flavonoid, tannin, carbohydrate, anthraquinone, saponins and protein [10, 11, 34].

Quantitative phytochemical analysis

Using the Folin-Ciocalteu technique, the total phenolic content of *Senna alata* extracts was determined [12] using certain adjustments, and the results were expressed as gallic acid (GA) equivalents in milligrams (mg) per gram of dry leaf extract (mg GAE/g).

Using the aluminum chloride method, the total flavonoid content was evaluated [13] using a few minor adjustments, and the total flavonoid were expressed as milligrams of quercetin equivalents (QE) per gram of extract from dried leaves (mg QE/g).

DPPH free radical scavenging assays

The previous approach was applied to evaluate the *Senna alata* leaves extract's capacity to scavenge DPPH free radicals [14]. To put it briefly, 2 mL of DPPH solution (60 µM) was added to 2 mL of ethanolic and aqueous extracts at varying concentrations (0.1-100 µg/ml). Next, incubate in the dark at 25 ºC for 30 minutes. For the positive control, ascorbic acid (AA) was used. [15, 16]. IC_{50} value of the sample containing plant extracts—that is, the

concentration required to scavenge 50% radicals was also determined. Each sample's free radical inhibition activity was calculated using the following formula:

DPPH free radical scavenging activity (%)

$$= [(A_{control\ 517} - A_{Sample\ 517})/ A_{control\ 517}] \times 100$$

Where, $A_{control\ 517}$ is the absorbance control $A_{sample\ 517}$ is the plant extract sample absorbance

In vitro thrombolytic activity

For assessing the clot-dissolving activity of *Senna alata*, a plant extract, compared to positive (Streptokinase) and negative control (water). Blood samples water collected from 21 healthy volunteers and distributed into pre-weighed micro centrifuge tubes. Following clot formation, serum was extracted, and the weight of the clot was measured. *Senna alata* extract, streptokinase, and water were added separately to different tubes. Following 90 minutes incubation, after removing any fluid that had leaked, the tubes underwent another weight measurement. As a percentage of clot lysis, the weight difference between before and after clot lysis was computed.

The experimental setup allowed the evaluation of *Senna alata* effectiveness in dissolving blood clots and comparing it to the controls [17].

$$\% \text{ clot lysis} = \frac{W_3 - W_2}{W_2 - W_1} X\ 100$$

Where, Clot weight = W_2-W_1

W_1 = weight of tube alone, W_2 = weight of clot containing tube, W_3 = final weight of tube with test

Brine Shrimp Lethality Bioassay

In this experiment, the lethality test for brine shrimp was utilized to assess the cytotoxic potential of a plant extract [18]. Six different concentrations of the extract were tested, ranging from 800 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml. After a 24-hour exposure, the number of surviving shrimps was recorded. Larvae showing no movement were considered dead. Negative control using Dimethyl sulfoxide and a Vincristine sulfate as reference standard were included. To make sure famine was not the cause of the observed death, they were compared with control group.

The toxicity of the plant exracts was determined by calculating the median lethal concentration (LC_{50}) using probit analysis, as described by Finney (Singleton & Rossi, 1965). The Brine Shrimp lethality bioassay offers several advantages, including its rapidity, low cost, simplicity, and the ability to use a large number of organisms for statistical validation. It also requires minimal sample volume (2-20 mg or less) and does not necessitate animal serum, which is typically needed for other cytotoxicity assays.

Mortality % = (No. of Dead larvae / Total no. of Larvae) x 100%

Antibacterial activity

Evaluation of antibacterial activity

To evaluate antibacterial activity, the agar well method was employed. In the method test, organisms were gathered, isolated as pure cultures, and standardized using the 0.5 M Mac-Farland standard [19].

Microorganism culture

It was decided that selectable microorganisms may be used for the investigation of antibacterial characteristics. The ATCC culture was obtained from the MMIHS laboratory, while the clinical isolates were obtained from the Natural Product Research Laboratory, Thapathali, Kathmandu. The creatures being assessed were:

Gram positive: *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC6051)

Gram negative: *E.coli* (ATCC 8739), *Klebsiellapneumoniae* (ATCC700603)

Mueller Hilton Agar (MHA) petri plates that had just been made were used to cultivate the obtained bacteria. The organism was allowed to develop in peptone water broth for the purpose of standardization prior to the test. Before being injected into the petri plates, the organisms in peptone water broth were cultured for 4-5 hours.

Standard Preparation

The antimicrobial evaluation standard employed in the experiment consisted of (320, 160, 80, 40) µg/ml of a solution of Gentamycin and Azithromycin dissolved in 1% DMSO.

Sample preparation

Different concentration of Normal extract solution (320, 160, 80, 40) µg/ml was dissolved in DMSO solution.

Test Procedure

The bore well diffusion method was employed for the test. According to the manufacturer's directions, MHA agar was made. The agar was prepared and 15 minutes of autoclaving at 15 pounds of pressure. In a sterile laminar hood, sterile petri dishes were filled with agar. The sun could set. For the test method, bores were prepared using an 8mm well borer. Using a sanitized swab stick, the bacteria were extracted from the peptone water broth and swabbed in the petri plates. Using a micropipette with sterile tips, 100 g/L of the extracts were collected. For 5 different concentrations of the test extracts, 5 holes were punched into each plate. Standard and blank underwent a similar process. Following a 24-hour incubation period, the zones of inhibition were identified on the plates.

Statistical analysis

The mean ± standard deviation of the data was displayed. Using the regression line equation in Microsoft Excel (2007), the standard calibration curve for gallic acid and quercetin was created to estimate the concentration of phenolic and flavonoid compounds. The findings were displayed in the table and picture. SPSS version 16 was used to do the statistical analysis.

RESULTS

Qualitative phytochemical analysis

Numerous phytoconstituents, including tannin, flavonoids, saponin, carbohydrates, terpenoids, and cardiac glycosides, were found in methanol extracts using qualitative phytochemical screening; these results are shown in Table 1.

Table 1: Phytochemical screening of water and ethanol extract of *Senna alata leaves*.

Phytochemical constituents	Specific tests	Result
		Methanol extract
Alkaloid	Mayer's test	-
	Wagner test	-
	Hager’s test	-
	Dragendroff’s test	-
Carbohydrate	Molish's test	+
	Benedict's test	+
Glycoside	Modified Borntrager test (Anthraquinones)	-
	Killer killiani test (Cardiac glycoside)	+
Saponin	Foam test	+
Phenol	Ferric chloride test	+
Flavonoid	Alkaline reagent test	+
	Shinoda test	+
	Zn-HCl test	+
Tannin	Gelatin test	+
	Ferric Chloride test	+
Terpenoid	Salkowaski test	-
	Copper acetate test	-

+: Presence, -: Absence

GC-MS analysis

GC-MS analysis of the *Senna alata* methanol leaf extracts revealed that the presence of the 3-methylmannoside, neophytadiene, squalene, campesterol, stigmasterol, alpha-tocospiro (Table 2).

Table 2: GC –MS analysis

S.N	Name of Compound	Molecular formula	Reported Activity
1	3-Methylmannoside	$C_7H_{14}O_6$	Plant growth regulator/ regulate plant growth by modulating glycoconjugation to lectins in plants
2	Neophytadiene	$C_{20}H_{38}$	Antimicrobial, additive for liquid cigarette
3	Squalene	$C_{30}H_{50}$	Antioxidant
4	Campesterol	$C_{28}H_{48}O$	Anticancer, Antimicrobial, anti-inflammatory
5	Stigmasterol	$C_{29}H_{48}O$	Anticancer, Antiinflammatory
6	Alpha.Tocospiro	$C_{29}H_{50}O_4$	Cytotoxicity against human A549 cells by SRB assay.
			Antimicrobacterial activity against Mycobacterium tuberculosis H37Rv

Quantitative Phytochemical analysis

The methanol leaf extracts of *Senna alata* exhibited the highest percentage of extraction yield (5.92%). Highest phenol content was observed in methanol extract (46.36±4.5) mg GAE/gm dry extract weight while highest flavonoid content was found in methanol extract (480.4±3.055) mg QE/gm dry extract weight (Table 3).

Table 3: Extraction yield (%) of three solvents of *Senna alata* leaves, total phenolic and flavonoids content.

Extract	Extraction yield (%)	Phenols (mg GAE/g dry extract weight)	Flavonoids (mg QE/g dry extract weight)
Methanol extract	5.92	46.36±4.5	480.4±3.055
Hexane extract	1.54	44.89±4.49	476.17±4.33
Ethyl acetate	0.78	40.66±0.36	435.77±4.81
Values calculated from the mean of three times experiment and represented as mean ± S.D			

DPPH radical scavenging activity

Compared to conventional ascorbic acid, the methanol extracts of *Senna alata* leaves (IC_{50} value 29.81 µg/mL) demonstrated good DPPH free radical scavenging action (Table 4, Fig. 1).

Table 4: IC_{50} value and DPPH free radical scavenging activity of both *Senna alata* extracts at varying concentrations.

Extract/ Standard	% activity of DPPH scavenging					IC_{50} µg/mL
	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL	
Methanol extract	15.13±0.0093	24.65±0.0063	29.28±0.0094	37.33±0.0290	42.83±0.0049	29.81
Ascorbic acid	41.19±0.0090	61.69±0.0012	75.59±0.0050	92.56±0.0050	97.07±0.0080	6.12
Values calculated from the mean of three times experiment and represented as mean ± standard deviation (n=3).						

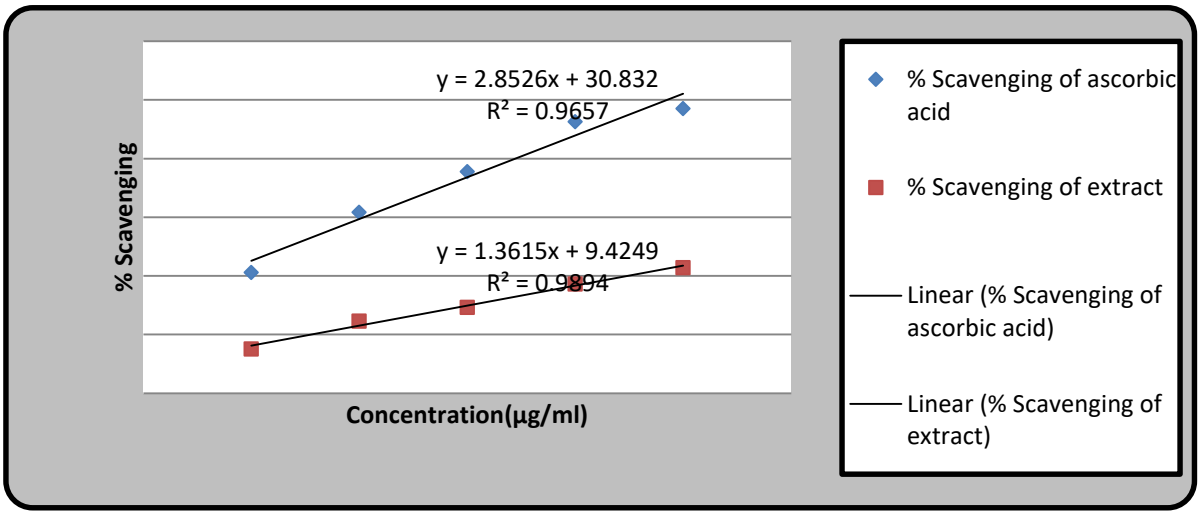

Fig. 1. Antioxidant activity by using DPPH.

Thrombolytic activity

This study found that the percentage of clot lysis was 7.89% at 10 mg/ml and 10.13 % at 25 mg/ml. In a similar vein, clot lysis with conventional streptokinase was found to be 40.77% (Table 5, Fig. 2).

Table 5: Percentage clot lysis of extract.

S.N	Concentration(mg/ml)	% clot lysis
Extract 1	10	7.89
Extract 2	25	10.13
Streptokinase	30,000 I.U	40.77
Negative control	D/W	2.03

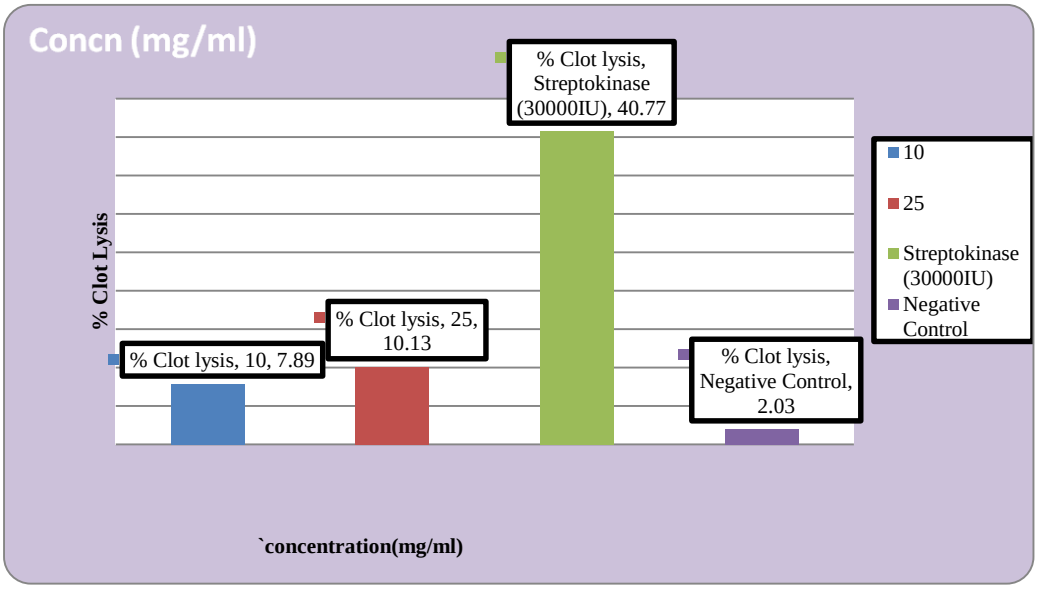

Fig. 2. Thrombolytic activity of the extract.

Anti-inflammatory activity

Human red blood cell (HRBC) membrane stabilizing method assessed the in vivo anti-inflammatory properties of *Senna alata* extracts. As a standard, Diclofenac Sodium was used. The results obtained by studying in vivo anti-inflammatory activity are tabulated in Table 6 (Fig. 3, 4, 5).

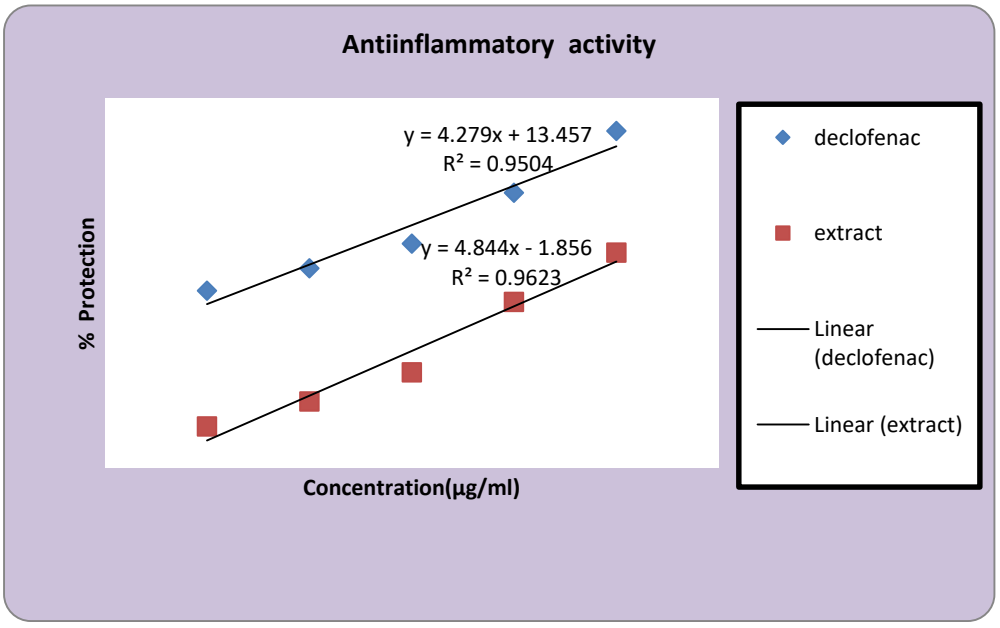

Fig. 3. Anti-inflammatory activity of standard and extract.

Table 6: Percentage protection and percentage hemolysis of extract and standard.

Concentration(µg/ml)	Percentage Protection		% hemolysis	
	Diclofenac	Extract	Diclofenac	Extract
10	19.21	4.5	80.79	95.46
20	21.62	7.4	78.38	92.75
40	24.32	10.32	75.68	89.62
80	29.81	18.16	70.19	77.90
100	36.51	23.32	63.49	75.17

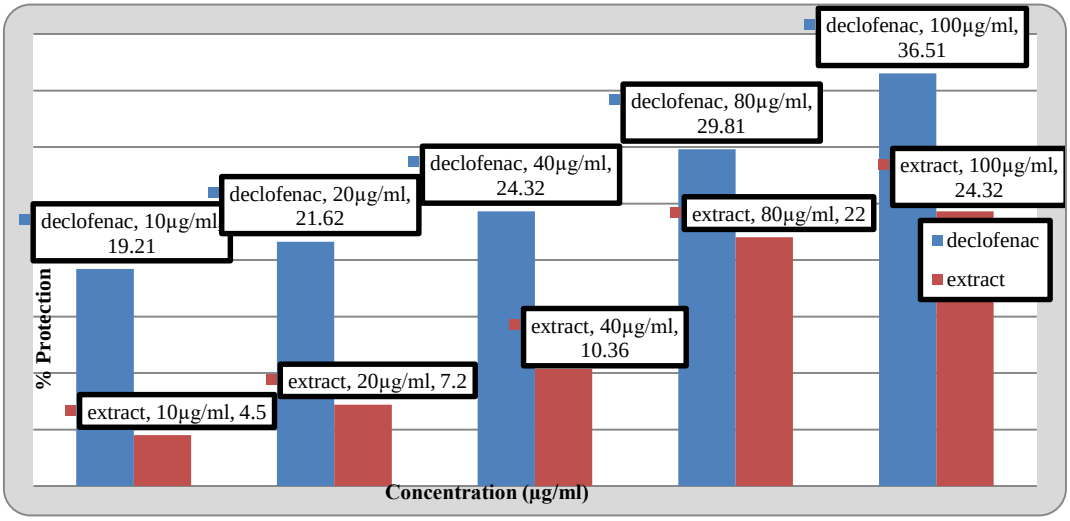

Fig. 4. Anti-inflammatory activity of the *Senna alata* extract.

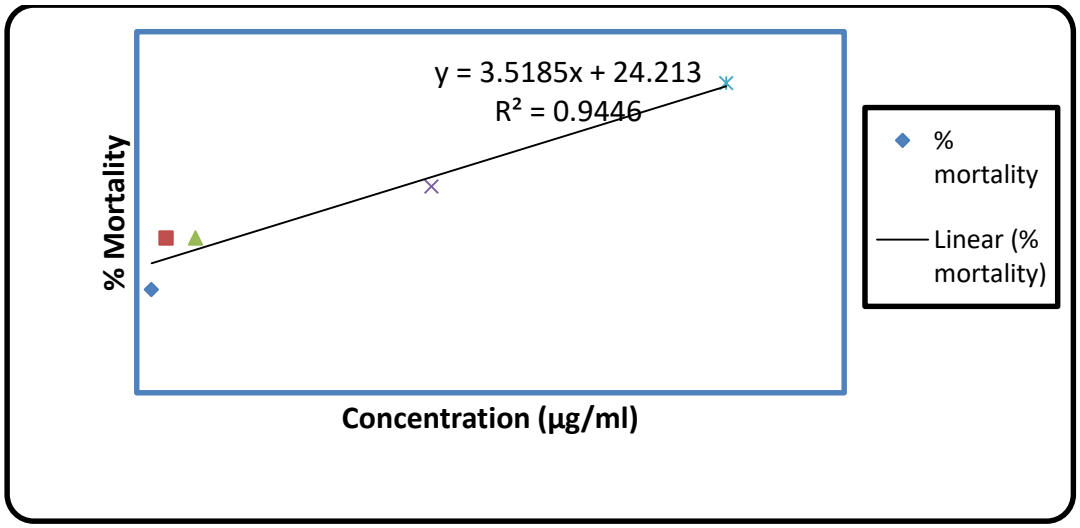

Fig. 5. Cytotoxic activity of vincristine Sulphate

Cytotoxic activity

Vincristine sulfate had an LC_{50} value of 7.32 (µg/ml), while *Senna alata*'s methanolic extract had an LC_{50} value of 767.85 (µg/ml) (Table 7, 8, 9) (Fig. 5, 6).

Table 7: EC50 values for Diclofenac and extract.

Name	EC50
Diclofenac sodium	8.54
Extract	9.93

Table 8: Percentage mortality by standard Vincristine Sulphate

Concn (µg/ml)	% mortality	LC_{50} value
0.25	20	7.32
0.5	30	
1	30	
5	40	
10	60	

Table 9: Percentage mortality of brine shrimp by extract.

Concentration (µg/ml)	% Mortality	LC_{50} (µg/ml)
50	0	767.85
100	0	
200	10	
400	30	
800	50	

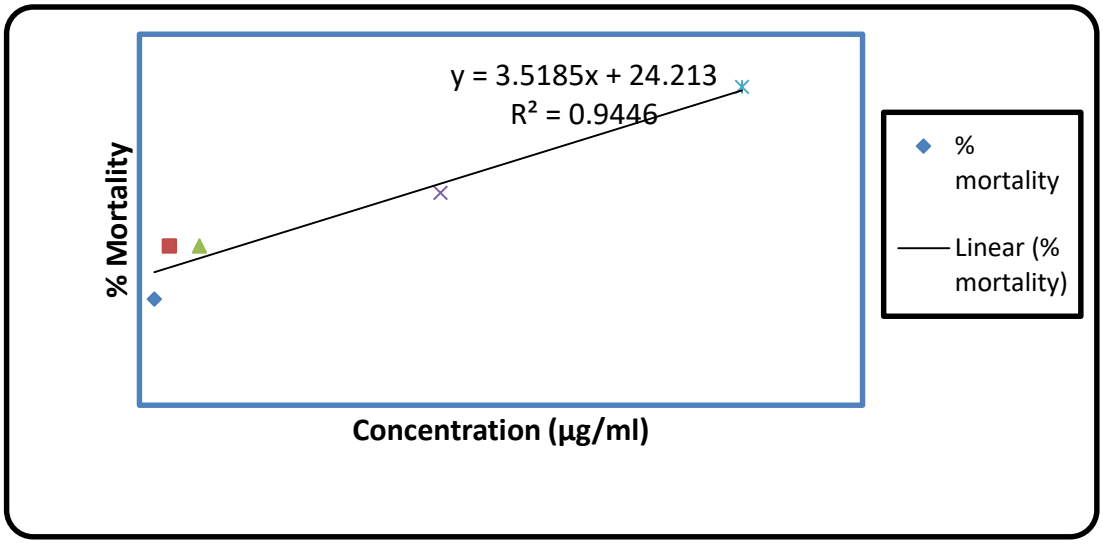

Fig. 5. Cytotoxic activity of vincristine Sulphate

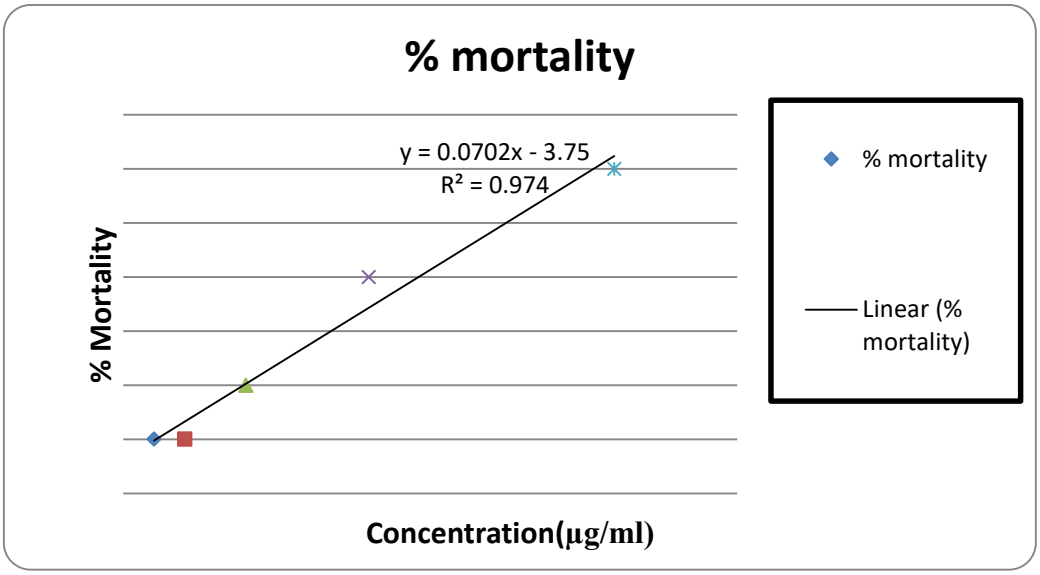

Fig. 6. Cytotoxic activity of *Senna alata* extract

Antibacterial activity

Table 10 displays the methanolic extract's antibacterial activity. While the extract does not demonstrate activity against *E. coli*, it does demonstrate activity against *S. aureus*, *B. subtilis*, and *K. pneumoniae* (Fig. 7, 8, 9).

Table 10 Antibacterial activity

Sample	Concn (µg/ml)	Inhibition zones of antibacterial screening (mm)			
		S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*
Azithromycin	40	5	10	-	-
	80	7	12	-	-
	160	12	19	-	-
	320	14	21	-	-
Gentamycin	40	-	-	3	8
	80	-	-	3	9
	160	-	-	4	13
	320	-	-	7	15
Extract	40	2	-	-	-
	80	3	1	-	-
	160	4	2	1	-
	320	6	4	2	-

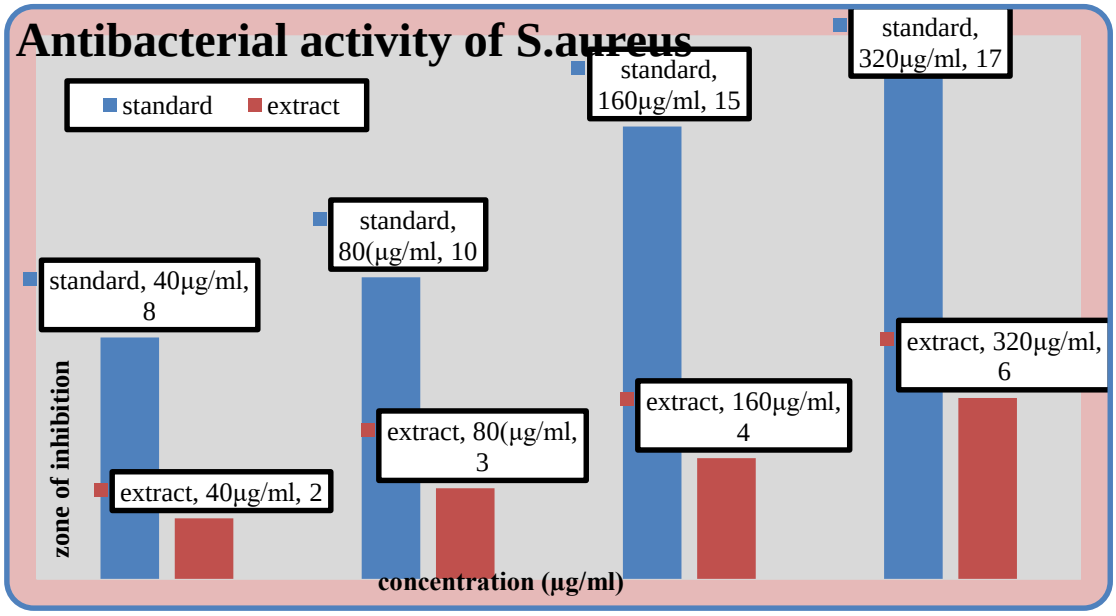

Fig. 7. ***Antibacterial activity of Senna alata extract against S.aureus***

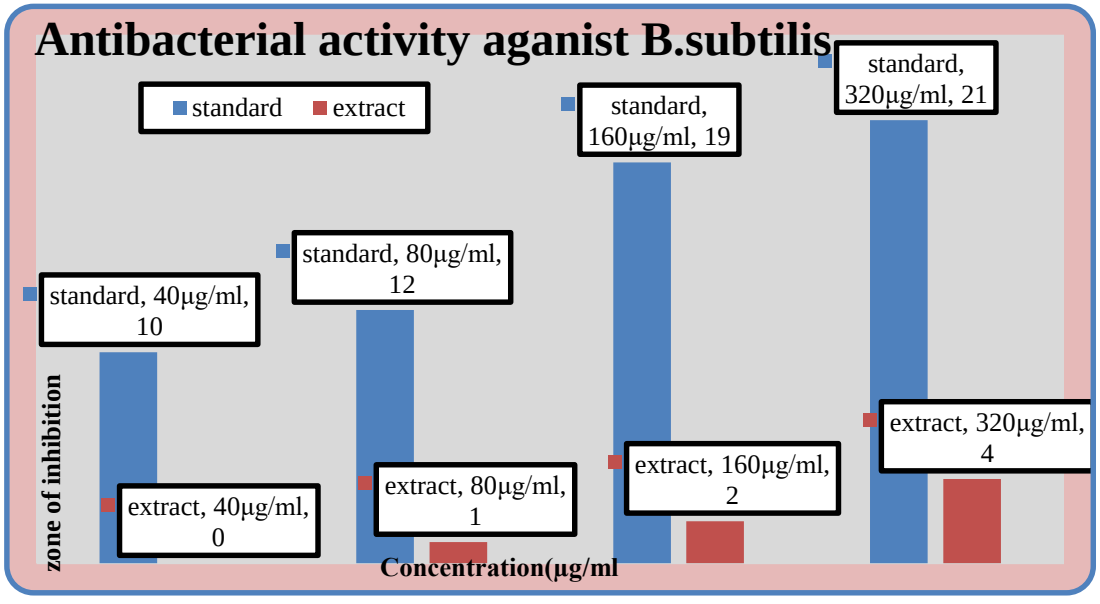

Fig. 8. Antibacterial activityof *Senna alata* extract against *B.subtilis.*

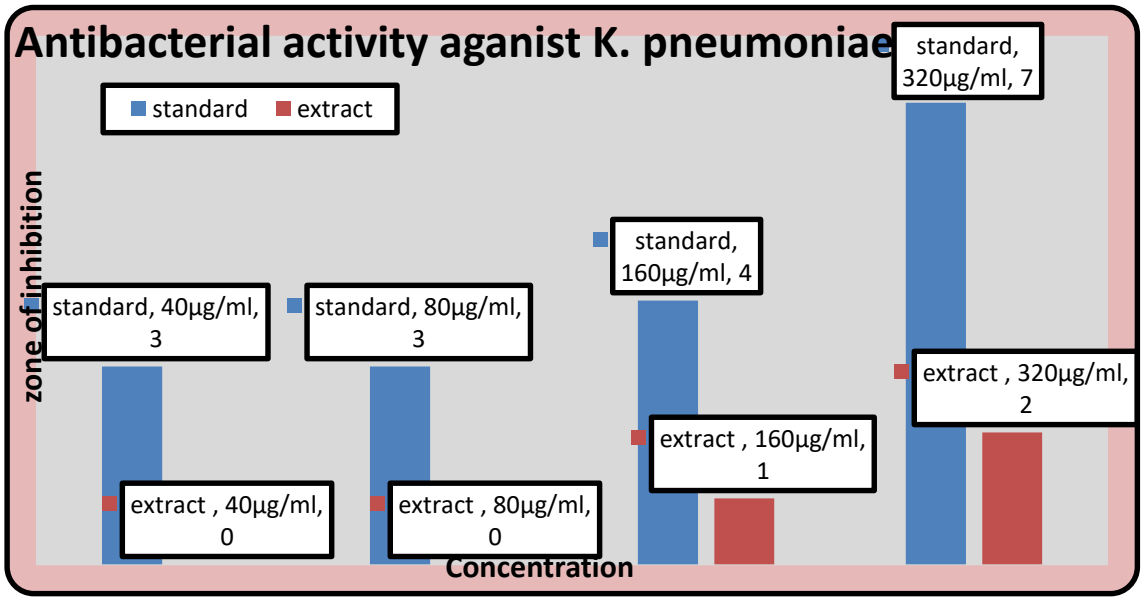

Fig. 9. Antibacterial activity of *Senna alata* extract against *K .pneumoniae*.

DISCUSSION

It is widely accepted that natural products are the most valuable source of lead compounds for innovative drug development in the pharmaceutical industry. Bioactive plant components are used as drug candidates or drug substitutes to treat various human diseases [20]. The selection of *Senna alata for* this communication was based on its limited scientific research, and traditional and ethnomedicinal uses. *In vitro* antioxidant, *antibacterial*, *in vitro* anti-inflammatory, thrombolytic, cytotoxic activities, phytochemical profiling and *Senna alata* leafmethanolic extract was subjected to GC-MS analysis.

The percentage yield was highest in methanol, at 5.92%. In a previous study, the methanol extract of Senna alata yielded 8.32% [21]. This was mostly impacted by various cultivation circumstances, including climate, plant location, and harvest times. The solvent's polarity has an impact on the phytochemicals that are recovered in the extracts.

The phytochemical analysis of *Senna alata* extracts showed the occurrence of a variety of chemical components that may have different pharmacological effects, such as flavonoids, carbohydrates, tannins, saponins, and cardiac glycoside. An earlier investigation found that the *Senna alata* plant's root and leaf extracts have antimicrobial properties in Nigeria revealing that saponins, alkaloids, flavonoids, anthraquinone, tannins, phenols, and glycosides are present in *Senna alata* [18]. Because they contain phenols and flavonoids, plant secondary metabolites have anti-inflammatory and antioxidant properties. They also have a favorable correlation with as antibacterial, cytotoxicity, and anti-inflammatory activity [18, 31].

The total phenolic content was determined using Folin-Ciocalteu's technique in using a standard agent as gallic acid. The leaves of *Senna alata* had the highest quantity of total phenols (42.76±2.13 mg GAE/g dry extract weight) in the methanol extract. Using quercetin as a reference, the

total flavonoid concentration was determined using the aluminum chloride colorimetric test. Of the two methanol extracts with flavonoid content, this one has a high concentration of flavonoids (34.97±2.86 mg QE/g dry extract weight). It demonstrates how important a role the solvent system plays in the solubility of various chemical components. It has been demonstrated that higher polarity solvents remove phenolic chemicals from the entire plant more effectively than lower polarity solvents [22]. This result agrees with the previous study [23]. The similar previous study performed on the methanol extract of this plant showed 41.6±0.41 mg GAE/g and 31.9±0.63 QE/g of the dried extract [21].

The various samples' DPPH radical scavenging ability was tested at various doses (at 5, 10, 15, 20, and 25 µg/ml) methanol extract revealed the concentration-dependent radical scavenging activity. Ascorbic acid's IC_{50} value in the DPPH scavenging method was 6.62 (µg/ml), while the plant extract's IC_{50} value was 29.81 (µg/ml). Previous study conducted by J. Sujatha, and S. Asokan showed that the IC_{50} value was 24.56 µg/ml [24]. According to this study, the IC_{50} value decreased as the phenolic and flavonoid concentration increased. The plant samples' antioxidant activity could be attributed to the presence of these chemical ingredients [25]. It has been demonstrated that plants with flavonoid and phenolic compounds can scavenge free radicals in living things [24]. Plant metabolites known for their phenolic and flavonoid components are widely distributed and exhibit a variety of pharmacological properties, including antibacterial, antioxidant, hepatoprotective, antidiabetic, and antimutagenic properties [26, 27]. The majority of compounds classified as antioxidants are derived from plants as secondary metabolites, such as phenolic compounds (flavonoids, phenolic acids, tocopherols, etc.) [25]. Because they can scavenge reactive oxygen species such as superoxide free radicals, singlet oxygen, and hydroxyl radicals, phenolic compounds have the potential to be antioxidants [28, 32]. The many functional hydroxyl groups found in flavonoids mediate their antioxidant action by scavenging dangerous free radicals and chelating metal ions to prevent the generation of dangerous radicals that damage vital biomolecules. Lipid peroxidation is oxidative stress's most frequent side effect. Through a variety of mechanisms, flavonoids play a significant role in lipid peroxidation against oxidative damage [29, 33].

This study showed that the lethal concentration (LC_{50} value) for the *Senna alata* leaves extract was found to be 767.85 µg/ml and the highest mortality percentage was 30 % at the concentration of 800 µg/ml. In the earlier research carried out by M.A. Awal et al, it was found that the toxicity effect of ethanolic leaf and seed extract of *Cassia alata* and found promising activity, rated that LC_{50} value of 4.31µg/ml for seed and 5.29µg/ml for leaf [18]. The phytochemicals present in plants such as alkaloids, flavonoids are believed to have an anti-cancer activity that can inhibit either initiation or progression of the tumors. The absence of alkaloids may be causes for relatively lower value of cytotoxicity activities [35, 36].

In the anti-inflammatory activity, the percentage protection at 100µg/ml was found to be 36.51% and 23.32% for the standard drug Diclofenac sodium and extract respectively. It was discovered that the thrombolytic activity at a concentration of 10 mg/ml was 7.89% and at 25 mg/ml it was 10.13 %. In the earlier investigation carried out by Adnan Mannan et al., the extract of cassia seed showed 37.92% clot lysis when the amount of 100µl/ml [17].

The zone of inhibition's existence verifies the test substance's capacity to impede growth. The largest zone of inhibition was found to be against *Streptococcus aureus* i.e. 6mm and for *Bacillus subtilis* it was found to be 4 mm. In addition, the extract did not show any action against the E. *coli* and for *Klebsiella pneumoniae*, and it was found to be 2mm. In the previous study conducted by AA. Ogunjobi and M.A Abiala, the methanol extracts of *Senna alata* powder inhibited the growth of *Staphylococcus aureus*, and *Bacillus subtilis* with inhibition zone diameters of 15 mm and 12mm respectively [30].

The results of the current study on *Senna alata* included higher concentrations of phenolic and flavonoid components as well as potent thrombolytic, antibacterial, anti-inflammatory, and antioxidant properties. Considering that it might be a strong candidate for the creation of an innovative oral medicinal substance.

CONCLUSIONS

In summary, the methanol extraction of Senna alata leaves yielded substantial amounts of extract, along with a significant concentration of flavonoids and phenolic compounds exhibiting antioxidant activity. The GC-MS analysis identified 3-methylmannoside as having the highest area ratio in Senna alata. This study suggests potential antibacterial, thrombolytic, cytotoxic, and anti-inflammatory properties of the methanol extract. These findings support the need for further scientific validation and research to explore the therapeutic benefits of this medicinal plant in combating microorganisms, inflammatory diseases, and diseases related to harmful cell proliferation.

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Author contributions

DK and BR conceived and designed the experiments. DK, BP, PJ, and AA performed the experiments. BP, DPK, BR, and SP analyzed the data. DK, PJ, BP, and AA wrote the manuscript. BP, DPK, BR, and SP reviewed the manuscript. SP and BP critically revised the manuscript and provided intellectual input. DPK and BR supervised the project.

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Data Availability Statement

All relevant data is in the paper and any query regarding the findings of this study is obtainable from the corresponding author upon request.

Conflict of interest

The authors declare no conflict of interest.

Approval of the research protocol by an Institutional Reviewer Board and the approval number

The study was conducted with utmost care and showed no signs of endangering people or the environment. Ethical clearance was obtained from the Institute Review Committee of the Department of Pharmacy, Manmohan Memorial Institute of Health Sciences, Kathmandu (Approval No: MMIHS-BP-2018).

Informed Consent

Before participation, informed consent was properly obtained from the willing human blood donors. Additionally, municipal regulations were consulted to secure permission for research on the subject plant.

Registry and the Registration No. of the study/trial N/A

Animal Studies N/A

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Senna alata الكيمياء النباتية، ومضادات الأكسدة، والتحليل، ومضادات الالتهاب، والسمية الخلوية، والنشاط المضاد للبكتيريا، وتحليل الكتلة الطيفية للغاز

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ملخص

الهدف: تتمتع الأعشاب الطبية في نيبال بأهمية ثقافية كبيرة ولها استخدامات متعددة. يعتبر نبات السنا ألاتا، وهو نبات ينتمي إلى عائلة البقوليات، ذو قيمة عالية لخصائصه الجمالية والعلاجية. وكان هدف الدراسة هو استخراج أوراق السنا ألاتا من خلال عدة نقعات بالمذيبات.

الطرق: لتقييم الكيمياء النباتية، ومستويات الفينول الكلية والفلافونويد، والخصائص المضادة للأكسدة في المختبر، والتأثيرات المضادة للالتهابات، والسمية الخلوية، والإمكانات المضادة للتخثر، والنشاط المضاد للبكتيريا، استخدمت هذه الدراسة مجموعة متنوعة من المنهجيات.

النتائج: تم تحديد القيم الاستخراجية للسنا ألاتا على أنها 1.58٪ و 0.78٪ و 5.92٪ في الهكسان، وأسيتات الإيثيل، والميثانول، على التوالي. كشف تحليل كروماتوغرافيا الغاز–مطياف الكتلة عن مركبات رئيسية مثل 3–ميثيل مانوسايد، ونيوفيتادين، وكامبيستيرول، وفيتامين هـ في مستخلص الأوراق. وقد أثبت الفحص الكيميائي النباتي النوعي محتوى التانين والكربوهيدرات والفلافونويدات والجليكوسيدات القلبية والجليكوسيدات والسابونين في مستخلص الميثانول. وكانت القيم الفينولية الكلية والفلافونويدية 46.36±4.5 ملغ / GAE جم و 480.4±3.055 / QE جم من المستخلص المجفف على التوالي. أظهر المستخلص أنشطة مضادة للأكسدة ومضادة للالتهابات بشكل ملحوظ، مع قيم IC50 29.81 و 9.93 على التوالي. وعلاوة على ذلك، فقد أظهر نشاطًا سامًا للخلايا بقيمة LC50 767.85 في اختبار الروبيان الملحي. من حيث النشاط التحللي للخثرة، أظهر المستخلص نسب انحلال الجلطات 7.89٪ و 10.13٪ عند تركيزات 10 ملغ / مل و 25 ملغ / مل على التوالي.

الاستنتاج: وفي الختام، أظهر المستخلص الميثانولي لنبات السنا ألاتا إمكانات علاجية، بما في ذلك التأثيرات المضادة للأكسدة والالتهابات والسمية الخلوية ومضادات التخثر والبكتيريا. كما أكد وجود العديد من المواد الكيميائية عن طريق تحليل كروماتوغرافيا الغاز–مطياف الكتلة على إمكانات النبات للاستخدام العلاجي.

الكلمات الدالة: *Senna alata*، الفحص الكيميائي النباتي، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات، النشاط المضاد للتخثر، النشاط السام للخلايا.

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